PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Colour-Physiology of Higher Crustacea. Part III

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Phil. Trans. R. Soc. Lond. B 1906 **198**, 1-16
doi: 10.1098/rstb.1906.0001

PHILOSOPHICAL TRANSACTIONS.

I. *The Colour-Physiology of Higher Crustacea.*—Part III.

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Communicated by Professor Sydney J. Hickson, *F.R.S.*

Received February 8,—Read February 23, 1905.

[Plates 1 and 2.]

Introduction.

The present is the third of a series of memoirs dealing with the Colour-Physiology of the Higher Crustacea.*

In this as in former joint-papers, though both authors hold themselves equally responsible for the whole work, the morphological investigations are more particularly due to Dr. Gamble, the physiological investigations to Mr. Keeble.

The research has been conducted in the following places :—Naples; the Zoological Department of the Manchester University; University College, Reading; and Mr. Keeble's laboratory at Trégastel, Brittany. A grant of £15 from the British Association has been of great assistance to the progress of the research. The paper consists of the following sections :—

I. The Histology of the Chromatophores of *Hippolyte* and *Crangon.*
II. The Occurrence and the Movements of Fat in the Chromatophores of *Hippolyte varians.*†
III. Sympathetic colouration.

I. *The Histology of the Chromatophores of* Hippolyte *and* Crangon.

Methods.—The most successful fixative is osmic acid (in water or in chromic acid). By its use the red and yellow pigments are preserved indefinitely, and the blue for several days.

* The two previous papers have appeared as "*Hippolyte varians:* A Study in Colour-change," 'Quart. Journ. Micros. Sci.,' vol. 43, 1900, and "The Colour-physiology of Higher Crustacea," 'Phil. Trans.,' B., vol. 196, 1903.

† The presence and movements of fat, and its disappearance in *Hippolyte varians* when kept in darkness, were the subjects of a brief note by us in the 'Zoologischer Anzeiger,' vol. 27, 1904, pp. 262–264.

Since the pigments of the living animal are in a *fluid* medium, it is necessary to place the intact animal in the fixative till coagulation occurs. With a 2-per-cent. watery osmic solution this takes 15 minutes, the specimen is then teased up with wooden or horn instruments, and the pieces required for examination left in the fixative for 2–3 hours. The employment of osmic acid has this additional advantage that it stains the fatty granules which occur in the chromatophores of *Hippolyte*, and thus admits of the relation between fat and pigment being ascertained. If it is required to remove the pigments, chrom-osmic (Fleming, strong formula) is used: or, in cases of more resistant pigments, decolorisation is effected by Mayer's chlorine method. Where osmic or osmic acid mixtures are used, the tissues are washed thoroughly after fixing, brought into weak glycerine and mounted in glycerine jelly.

Though the red and yellow pigments are, after fixation, fairly resistant to alcohol, they dissolve instantly when brought into contact with essential oils, and hence mounting in Canada balsam is to be avoided. If the pigments are thoroughly removed, the limits of the chromatophores become indistinct, otherwise the use of stains offers no serious difficulty.

The Chromatophores.—The distribution of the chromatophores of *Hippolyte* and of *Crangon* has been described in a former paper,* where also references to the literature are given. Their histology, however, is very incompletely known.

Figs. 1–17, Plates 1 and 2, serve to illustrate the following descriptions.

The mature chromatophores of *Hippolyte* and of *Crangon* are as a rule bi- or tri-chromatic. The pigments most constantly present are red and yellow, with blue in *Hippolyte varians* and violet in *Crangon*. In addition to these pigments, a reflecting substance†—white, yellow, or greenish—is often present and may, especially in larval (zœal) *Hippolyte*, form the sole constituent of the chromatophore. The pigments and the reflecting substance are mobile; now extending in the form of fine granules along the branches of the chromatophores, leaving the centres colourless, and again concentrating to so many separate masses at the centres of the chromatophore. The several pigments have their own rates of flow along the branches and react at different rates to a given stimulus.

Two other substances are associated with the chromatophores of *Hippolyte*: a nocturnal blue substance which, at night, envelopes the centre and extends along the branches; and a colourless granular fat, an account of which is given in a later section. The dermis of an adult prawn or shrimp exhibits not only these polychromatic structures, but also chromatophores in all stages of development, or upon occasion, of degeneration. Indeed, it is possible, by regulating the light-conditions under which the animal is kept, to call forth the development of chromatophores possessing pigments of any desired colour, *e.g.*, red only or red and

* 'Phil. Trans.,' B, vol. 196, 1903, p. 310.

† This substance is figured and described in our earlier paper (1903).

blue, yellow only, etc. It is possible, moreover, to obtain animals whose chromatophores are devoid of fat and practically depigmented.

Observations of the various chromatophores in the skin of an adult animal show that they do not follow one invariable line of development.

In *Hippolyte varians* the initial pigment may be red or yellow; or yet more frequently only the reflecting substance may be present. In the shrimp the initial pigment is red or yellow or violet. The succeeding stages are equally variable. The initial pigment in *Hippolyte* may increase in amount without the addition of a second. The chromatophore containing it may bud off a second, also bearing similar pigment, and so on, till the chromatophore comes to consist of as many as seven or eight pear-shaped centres, each containing pigment of one colour, and each communicating with a separate system of branches.

It happens more usually, however, that as the chromatophore develops, a second pigment and then a third is added, so that the centre consists of three compartments each of which contains only one of the three pigments present. The chromatophores of uniformly coloured varieties are very constant as to amount and kinds of pigment; whilst in parti-coloured varieties different parts of the body present, as would be expected, marked differences in these respects.

In contrast with the variable mode of pigment-development in the adult, that in the larvæ is singularly constant. In *Hippolyte varians*, for example, the zœa invariably possess a centralised system of chromatophores within which red pigment and a greenish reflecting substance are present. The yellow pigment only appears later; the blue after the red and before the yellow pigment, though, if the conditions under which the zœa are kept are unfavourable, it may make its appearance at the same time as, or even before, the red.

The order of occurrence of these pigments is probably capable of a simple explanation. As experiments described in Section III. show, red pigment is formed and yellow is destroyed in darkness, whilst yellow is formed and persists, and red may be destroyed in fair illumination. In the earliest stress of life the nervous chromatophoric mechanism is not fully functional, it is—to use a figure of speech—blind to the light. At this, the zœa stage, the red pigment arises, which though sensitive to light, is formed even in darkness; and as the nervous system of the chromatophores becomes sensitive to the light, yellow pigment begins to form, and, under certain circumstances, dominates the red.

In the *Crangon*-zœa, a claret-coloured pigment and a yellow reflecting substance are alone present; the red, yellow, and violet pigments appear later; in what manner we discuss later.

The structures within which these pigments are elaborated and distributed are of composite nature. They consist in adult prawns and shrimps of a series of nucleated compartments, pyriform toward the centre of the chromatophore, and drawn out peripherally into branched tubular trunks, terminating tree-like in the

intercellular spaces. The compartments are flat cells, or rather cœnocytes, arranged generally in two concentric series, central and peripheral. Along a given radius there is continuity between central and peripheral cells. When contracted to the centre, the pigment is contained in the central cell in the form of a band or chromatophore in the botanical sense; in the expanded chromatophore the pigment has passed to the peripheral cell and its branches.

The cytoplasm of these cells is differentiated in a manner unusual among animals into a firmer, more refractive ectoplasmic wall, and a viscous endoplasm (Plate 1, fig. 6). When the chromatophore expands, the endoplasm, carrying its pigment, streams out from the central cell into the branch cell; when the chromatophore contracts the streaming is reversed. In the former case the tubular branches become injected with protoplasm and pigment, in the latter the tubes show up empty and refractive, with here and there a nucleus pressed against their walls (Plate 1, fig. 6).

Simpler Types of Chromatophores.—A simpler type of chromatophore occurs in the dermis of *Hippolyte gaimardii*. The component cells vary in number from one to six, according to the grade of development (Plates 1 and 2, figs. 13–17). Hollow, non-nucleated branches are given off from the margin of each cell. In chromatophores of more than one cell, the cells are of two sizes, the smaller lodging the red pigment and the larger the yellow. We have not observed a blue pigment in this prawn. The branches of the chromatophores appear to fuse here and there with other branched cells of the connective tissue (*x*, fig. 16). After the chromatophore reaches the six-celled stage it becomes constricted and forms two distinct centres.

Hippolyte varians.—The parts of the chromatophores containing red and yellow pigment generally consist of more numerous, smaller cells, distinguishable as already described into central and peripheral groups. But simpler chromatophores also exist, consisting of three or even fewer, pyriform branched cells (fig. 7). From the branches of such cells small spherical vesicles containing blue pigment may be budded off; always as far as we have seen from branches in which red pigment runs.

All stages from the simple, one or at least few-celled, chromatophore up to the highly complex multicellular form already described, are to be met with in adult *Hippolyte varians* (figs. 7–10).

It remains to describe the other elements of the chromatophore of this species, viz., those which contain the diurnal and nocturnal blue pigments, and those in which the colourless fat are contained and distributed. In the young normal chromatophore no blue pigment is visible; but it appears soon after the red pigment begins to show mobility. The tubular branches in which the red pigment runs show themselves to be filled, when the red pigment is retracted to the centre, with a pale blue substance. In older chromatophores the outer of the two concentric series of cells constituting the centre comes to be occupied by this blue substance. When the red pigment is caused to contract, it either masks the blue completely or the latter may be seen lying at places in the cells opposite to those which lodge the red pigment. This

blue pigment is one source of the nocturnal blue colour, coming as it does to occupy the branches left vacant by the nocturnal and complete retraction of the red pigment. Beside the nocturnal blue pigment which occurs in all colour varieties of *Hippolyte varians*, brown and green *Hippolyte* contain a diurnal blue pigment lying in branched cells between those containing the red and yellow pigments.

The relation between nocturnal and diurnal blue substance appears to be as follows :—The blue substance arises from the red pigment. It makes its appearance along the same tracts or along tracts budded off from those containing the red pigment. As long as these tracts are continuous a reciprocal relation as to movement is maintained between blue and red pigments: when the red expands, the blue contracts, and *vice versâ*. In certain forms—green and brown *Hippolyte*—the blue system becomes separated off completely from the red and the yellow substance, and forming the diurnal blue pigment which, constrained no longer by the ebb and flow of the red, is free to occupy the branches of its own system. Insensitive to light, it remains permanently expanded, and, at most, may be only masked by the expanding red pigment.

The colourless fat occurs in cells either identical with or similar in position and branching to those which contain the blue substance. Thus the chromatophores of *Hippolyte varians* are structures of no little complexity and variability. They develop in the skin from an initial cell or from a small group of initial cells, and in any case soon come to consist of a group whose wider inner ends aggregate to form a central body, and whose narrow outer ends grow out to form a system of branching tubes, the walls of which bear nuclei along their course. In these cells the red and yellow pigments are contained. In additional branching cells, the blue pigment and the colourless fat are contained. From one chromatophore, new chromatophores may arise by unequal fission or by budding, and these new chromatophores may remain for a time in communication, as, for example, in the muscular tissues, or, as in the skin, soon separate from one another, though in both cases they act in unison in obedience to a given nervous stimulus.

Crangon vulgaris.—The development of the chromatophores and their pigments in the common shrimp offers many features in common with the process in *Hippolyte*, as may be seen by a comparison of figs 1–6 with figs. 7–11. The earliest stage in their developmental history consists of a single stellate cell of the connective tissue. Subsequently independent nuclei are found in the branch-cells, while the chromatophore-centre either remains single or becomes multiple (figs. 4, 5, 6). The hollow nature of the branch-cells when the pigment is retracted to the centre, is very evident in the chromatophores of the shrimp; and so also is the distinction between the mobile endoplasm and the stiff refractive wall of the cell-branches (fig. 6). The chromatophores of the shrimp attain a considerable size; and in this respect as well as in their rarity in the musculature, their peculiar violet pigment, and the absence of any special nocturnal colouration, offer a marked contrast to the chromatophores of *Hippolyte*.

II. *The Occurrence and the Movements of Fat in the Chromatophores of* Hippolyte varians.

In no case of sympathetic colouration is the significance of the phenomenon completely understood.

Two apparently opposed hypotheses have currency. These are the well-known hypothesis of protective or cryptic resemblance, and that which suggests that the pigments which give rise to the sympathetic colouration are non-significant as to their colour but play some unknown *rôle* in the economy of the animal which possesses them.

Neither point of view can be regarded as satisfactory. The first is incapable of direct experimental proof; the second is nothing but a negation and a guess.

To justify our statement that the protection hypothesis is incapable of direct proof, we may refer to the recent experiments of DI CESNOLA on *Mantis religiosa*. Green and brown forms of *Mantis religiosa* were placed on harmonising and on contrasting backgrounds. The latter were taken—by birds chiefly—and the former were left. The author concludes that the experiments "seem to show in a fairly convincing manner the value of protective coloration."

But the experiment supplies no proof that the pigments owe their origin to the need for protection. The pigments may have arisen and may exist either in obedience to the need for protection or in obedience to quite other needs.

Further, if sympathetic colouration has arisen in obedience to needs other than protection, it may have arisen in *exclusive* obedience to these other needs. The harmonising pigments, absorbing and reflecting rays of light similar to those reflected and absorbed by their environment, may have been produced by the direct reaction on the part of the animal protoplasm to this light. Protective colouration becomes then a happy chance, and not, so to speak, a deliberate adaptation.

Or, to put the matter in another way, protection in the ordinary sense becomes only a special case of a wider protection, involving not only a defence against enemies, but the establishment of a harmonious relation between the animal and the light-conditions of its environment.

Chromatic adaptation—adaptation in which the colour assumed by pigments has a definite relation to the "colour" of the light evoking pigment formation—is already known to occur.

In POULTON's brilliant researches it is shown that certain insects develop pigments of the same colour as the light which falls on them. More recently, GAIDUKOV (1902) has demonstrated the occurrence of "complementary chromatic adaptation" in certain of the blue-green algæ (*Oscillaria*). Filaments of this alga, transferred from one monochromatic light to another, *e.g.*, from red to blue, pass "through the intervening colours of the spectrum" to the colour complementary to that of the light

to which they are exposed. Thus, in light blue, the filaments become in the course of a few weeks red. The significance of the colour-change appears to be that assimilation is augmented, the added pigments increasing the amount of light-absorption. In any case there can be no question here of protection in the narrow sense.

Crustacea, such as *Hippolyte varians*, which exhibit sympathetic colouration with respect to the weeds among which they lie, show, like the weeds themselves, complementary chromatic relations to their light environment.

The colours of the animals may then have originated in one of three different ways: as a direct reaction on the part of the animal to the light, the reaction having no reference to the need for protection; or it may have been evolved solely in answer to such need; or it may represent a compromise between the two, the pigments themselves serving some function other than that of cryptic colouration, which function is capable of fulfilment by the pigments which have passed the censorship of protection. A first step toward the determination of which of the three above-stated alternatives is correct, consists in a thorough physiological and morphological investigation, without reference to these theories, of the chromatophore-system of the animals under consideration. If this has for a result the bringing to light of facts incompatible with the theory that the pigments have and have only a protective *rôle*, it then becomes necessary to cast about in order to ascertain what other function they possess.

We have taken the first step. In the course of our observations a number of facts have come to light which are difficult of interpretation in terms of protection. The nocturnal blue colouration which, as we have described, is assumed by *Hippolyte varians* and other Crustacea, cannot, as we conceive, be regarded as conferring any protection on these animals. *Hippolyte varians*, like all sympathetically coloured animals, is in the highest decree sedentary; as far as we know, it never migrates; nor would it, did it migrate through clear blue water, assume its nocturnal blue colour.

The pigmentary system of the higher Crustacea has a very ancient, definite, and uniform origin. It is laid down as a series of centralised chromatophores having constant relations to other organs.* The chromatophores of this primary system are already coloured, and the pigment shows periodic as well as light-induced movements of contraction and expansion, yet the pigments produce practically no optical effect. The ultimate chromatophore-system of the adult animal overlies and masks this primary system which, nevertheless, continues to persist and function.

The chromatophores of *Hippolyte* are not confined to situations where their pigments may be optically effective. They occur not only in the skin, but invest the liver and gut and follow the muscles in their courses.

These facts, though not necessarily invalidating the hypothesis of protection, make

* 'Phil. Trans.,' B, 1903, vol. 196, p. 320.

imperative the more difficult task of inquiring whether the chromatophore-system of so typically a sympathetically coloured animal as *Hippolyte* has not some quite unsuspected function.

The first possibility which we began to consider, and with which this section is concerned, was whether the chromatophores of *Hippolyte* may have some photosynthetic function; whether they may do for the animal what chlorophyll does for the plant, namely, synthesise carbohydrates or some other carbon-containing substance by the aid of radiant energy. An examination of the pigment shows that they contain carotin. Now carotin occurs in plant chloroplasts, though not as a constituent of chlorophyll. Moreover, according to KOHL's researches, carotin is capable of some measure of photosynthetic activity. Hence, if we may rely on the accuracy of KOHL's observations, the possibility that some photosynthetic activity is displayed by the crustacean pigments becomes almost a probability.

Our next step was to ascertain whether the chromatophores contain any plastic substances comparable with those manufactured by the chromatophores of plants. Now it is well known that the "first visible product of photosynthesis" in plants is generally starch, but that in certain monocotyledons and algæ, oil takes the place of starch. An examination of the contents of the chromatophores of *Hippolyte varians* and *Hippolyte viridis* showed (1904) that oil or fat is present in them, often in considerable quantities. As means of identification we use (1) the optical character of the granules; (2) the osmic acid reaction; (3) Sudan 3; and (4) Schlarlachrot (MICHAELIS methods).

We describe below the varying appearances and the behaviour of this chromatophore-fat, confining ourselves here to the argument as to its significance. The presence of fat being demonstrated in the chromatophores of *Hippolyte*, the questions arise: Is this fat a photosynthetic product or is it derived from the food? Is it a plastic substance temporarily stored in the chromatophores or is it a specialised substance from which the pigments themselves are elaborated?

We endeavoured to get the answer to the question as to the origin of the fat in the chromatophores by adopting the method employed by botanists, viz., that of causing depletion by darkness, then observing whether exposure to light brings about a formation of starch or oil. Batches of *Hippolyte* were divided into comparable lots; one lot was killed and the state of the chromatophores with respect to fat examined, a second lot was placed in darkness without food, and a third in darkness with food. The results of such depletion experiments—experiments which we have repeated again and again during the past two years—show that in starved animals in the dark the chromatophores gradually become depleted of fat till finally no trace is left. Unfortunately, the time taken by this depletion process is very variable, depending, no doubt, on the age and on the general state of nutrition of the animal, and on the quantity of fat in the chromatophores at the beginning of the experiment. In some cases in small animals all trace of fat is lost after four days;

in larger animals 6–8 days suffice, whilst in fully developed animals 14 days may not be long enough to insure this result. The depletion, beside taking place at very different rates in different animals, takes place also at different rates in different parts of the body. The carapace-chromatophores are the first to lose their fat, next those of the tail, and those of the telson and of the antennæ among the last.

As we show above, the fat is not mixed up with the pigments. It is carried in quite distinct compartments. The compartments of the chromatophore which contain the pigments do not give any fatty reaction whatever. Fat and pigments exist side by side in different parts of the organ which we call the chromatophore. When fat-depletion is achieved, the fat-containing elements of the chromatophore present a clear vacuolated appearance. In the stage immediately preceding this, the endoplasm of these elements takes on a pale uniform blue-black colour with osmic acid.

There is no doubt then that the fat of the chromatophores is a plastic (reserve) substance, and that it is yielded up to other tissues of the body when food supplies are lacking. That the fat is not used up during the sojourn in darkness in the manufacture of pigment is certain, for, as we describe later, the pigments decrease rather than increase in darkness.

We now turn to the more important question, the origin of the chromatophore-fat. Is it derived photosynthetically or from the food?

The experimental evidence which we have obtained points to the origin of fat by photosynthesis, but we wish before giving this evidence to state that on the principle that there should always be a direct proportion between weight of evidence and gravity of subject we do not consider our evidence sufficient. If we thought that it might be made stronger by repetition of the experiments, we should have delayed publication till such repetition could have been made. But as a matter of fact, we have repeated the experiments so often that we are convinced that our present methods can lead to no more certain result, and this because of the inevitable sources of error in our method of experiment. As postulated above, if fat-depleted animals brought into the light show a re-formation of fat in the chromatophores, we shall have made a large step toward the discovery of a photosynthetic process in the animal kingdom. But for the final step it will be necessary to prove that this fat is not derived from the liver, blood, or some other tissue.

We have stated, however, that there is a great variability in the rate of depletion of different animals even of similar sizes and colour. Here, then, is our first source of error. Though our controls taken from darkness are free from fat, we have no certainty that the similar animals which are to be submitted to the crucial test of light exposure are similarly free from fat. The only means of eliminating this source of error would be to apply the statistical method, to work with large numbers of animals. This is a sheer impossibility even for two workers, yet this is the

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method which, in the future, will have to be adopted for these and similar problems—a biometrical experimental physiology.

If we seek to make quite sure of depletion by leaving the animals still longer in the dark, their pigments undergo marked changes. The yellow disappearing altogether, the red persists for a time, but finally disappears, leaving the chromatophores as colourless vacuolated bodies, almost devoid alike of fat and of pigment (fig. 20).

Having discharged our duty in criticising the value of our results we will turn to these results themselves. Table I. is an example of a considerable number of experiments. Young starved specimens of *Hippolyte varians* show a considerable loss of fat after five days' darkness, whilst light-starved animals show possibly some, but certainly less loss. After six days, animals starved in darkness show complete depletion, whilst light-starved controls still contain a fair amount of fat in their chromatophores. Dark-starved animals, comparable with the above, are exposed after six days of darkness to a fairly bright light without being fed (from 11.30 A.M. to 6.30 P.M.). Examination then shows in one specimen a fair and in another a large quantity of fat.

TABLE I.—*Depletion of Fat* of Chromatophores *in Darkness. Hippolyte varians.* TRÉGASTEL, August–September, 1904.

Date.	Dark-starved animals.	Light-starved animals.	Dark-starved then exposed to bright light.	Light-starved then exposed to bright light.	Dark-fed.
Aug. 30. Fresh-caught animals; sample showed fair amount of fat in chromatophores.	—	—	—	—	—
Sept. 4. (5 days from beginning of experiment.)	1 fair amount of fat. 1 small amount of fat.	1 fair amount of fat. 1 fair amount of fat.	—	—	—
Sept. 5. 6 days...	1 no fat.	1 fair amount of fat.	1 fair amount of fat. 1 large quantity of fat.	1 some fat. 2 much fat.	1 much fat. 1 much fat.
Sept. 6. 7 days...	1 most chromatophores free from fat, here and there fat in the centres.	—	1 some small amount of fat (pigments gone).	1 Only traces of fat.	—

The experiment is repeated on the following day—after seven days' darkness—and the fat after light-exposure (12–5.30 P.M.) is found to be but small in amount. It is to be noted, however, that in these animals the pigments have almost disappeared. Other controls maintained in the dark but fed show, after six days, large quantities of fat in the chromatophores. This, however, does not necessarily indicate that the fat of the chromatophores is derived from the food. For with a plentiful supply of nourishment there is no reason why the reserves of fat in the chromatophores should be withdrawn; nor, since the formation of pigment falls off in darkness, will the reserves be used up locally.

One of the most unexpected results of our observations with reference to chromatophore-fat is that this substance is mobile like the pigments themselves. It occurs now concentrated at the centre of the chromatophore, now running out from the centre along coarse special branches, and again spreading throughout the finer twigs of these branches, producing the appearance of a delicate reticulum, or as punctate areolations confined to the flat supra-epithelial spaces in which the finest branches of the chromatophores terminate.

The mobility of the fat like that of the pigments is determined by the influence of light. In darkness the fat of the chromatophores is aggregated as a flat granular mass at the centre. Here it lies in flat plate-like elements which are below those containing the yellow and the red. The order of these elements is, uppermost yellow, next red, next blue and fat. In light, even in excessively dim light, the fat is carried up by the endoplasmic stream first into the stem-like branches which run out from the flat centre and then along these into their finest terminations. Figs. 18–20, illustrating the various positions taken up by the fat, are given in Plate 2, and these figures also serve to illustrate the steps of fat-depletion which may occur in darkness.

The reserves of fat are distributed during starvation in two ways. Those of the liver are transferred directly into the gut, along the liver-duct, occasioning for five or more days large quantities of ejecta, while the other fat-reserves pass into the blood-corpuscles in the blood spaces about the chromatophores. The corpuscles become laden with fine granules that give a characteristic reaction with osmic acid.

III. *Sympathetic Colouration.*

We have shown elsewhere that the adult *Hippolyte varians* possesses to a remarkably small degree the power of responding sympathetically to colour-changes of its environment. A certain percentage of animals, when transferred from weed of one colour to that of another, remain refractory; some assume, but only with slowness, the colour of the weed on which they lie. A week or more is required to convert a green *Hippolyte* into a brown.*

* See 'Quart. Journ. Micros. Sci.,' 1900, p. 614.

We have been able during the past summer to examine animals at different stages of development with respect to their powers of sympathetic colour-change.

Medium sized animals (see Table V.), of the red-lined colour-form, placed on green weed (*Zostera*), show distinctly more capacity for sympathetic colour-change than adults. Half remain unchanged during the period of the experiment (11 days), one-third of the total number undergo complete colour-change from red to green in about eight days, and the remainder halt in intermediate stages, *e.g.*, as speckled yellow, yellow-green, or dull green. The experiment shows that though colour-change is slow, yet that it is neither so slow nor so restricted as in adults.

Young transparent and nearly colourless *Hippolyte* behave in a very different manner. With them the colour-change is as surprising in its rapidity as it is in its slowness with adults.

Tables II. and III. record our experiments with these young fairly transparent animals. Placed on red-brown weed sympathetic colouration is observable in several animals on the following day, and in 11 out of 17 after two days. Similar originally transparent animals become green on green weed in the course of a day (Table IV.).

Nothing can be more impressive than to see the hue of the weeds among which they lie, rapidly stealing over the animals. Not the least extraordinary aspect of the matter is that the pigments which produce the sympathetic colouration are not already present when the experiment begins. They have to be produced and properly distributed. Whereas in the slow reacting adult, rich stores of pigments are present, and present in a highly mobile state, nevertheless such animals only react grudgingly to the changed environment.

The very young animals not only take on with great readiness the colour of their surroundings, but almost as readily change their colour when that of their surroundings changes. For example, colourless *Hippolyte* having been put on green weed became green in three days (Table I.). These green animals were then placed on brown weed (Table IV.), and in three days three out of six survivors became brown.

In the green state the yellow pigment was well developed, the blue fairly, and the red but poorly developed. In the brown state red was considerably increased and the blue decreased.

We conclude that, its pelagic life passed, *Hippolyte*, at the time of settling down on the weeds of the laminarian zone, possesses in a high degree the power of sympathetic chromatic adaptation. A day or so suffices to call forth the formation of the requisite pigments. At this early stage of life, change of habitat is not fatal to harmony of colour between the animal and its surroundings; such a change, leading rapidly to re-formation and re-adjustment of pigments, is followed by sympathetic colour-change. But as age comes on, elasticity is lost. The chromatophore system becomes more and more stereotyped, and should the colour of the environment change, either no alteration or at most a perfunctory alteration is produced in the

distribution of the constituent pigments. They are too much the consequences of the past which made them to be readily amenable to present influences. The colouration has become a habit.

These facts are closely analogous to the results of Professor POULTON's observations (1903) on the susceptibility of lepidopterous larvæ to the colours of their surroundings. Not only do the caterpillars of such sensitive forms as *Gastropacha quercifolia*, *Odontoptera bidentata*, and *Amphidasis betularia* reflect as it were the colouration of their evironment, but, in addition, they exhibit a specially high degree of susceptibility in their early larval stages, becoming less responsive in the later period of larval life, and, indeed, in some cases ceasing altogether to react to change of their environment by sympathetic colour-change.

TABLE II.—*Sympathetic Colouration. Hippolyte varians.* TRÉGASTEL, 1904. Animals used in the experiment: *young*, about 5–6 millims., *colourless, or pale-lined.*

July 26, put on RED-BROWN WEED.		Colour of the animals.	On GREEN WEED, July 26.		
July 27.	July 28.		July 27.	July 28.	July 29.
2	11	Red	1	1	1
1	—	Pink	1	1	1
3	—	Brown	—	—	—
1	—	Yellow-brown	—	—	—
1	1	Colourless	2	1	1
4 + 2*	2	Sundry	—	—	—
2	3	Yellow	1	—	—
—	—	Yellow-green	1	—	—
—	—	Pale green	5	6	—
1	—	Green	1	2	7

* Escaped observation on 27th.

The table shows that out of 15 colourless or pale-lined *Hippolyte* 11 had become red after two days' association with red-brown weed; that 8 out of 12 had become green on green weed in the same time.

TABLE III.—*Sympathetic Colouration. Hippolyte varians.* TRÉGASTEL, 1904. Animals used in the experiment: *young, colourless* to the naked eye; about 6 millims. in length. Maintained in glass vessels in a circulation of water.

July 16	6 *colourless* Hippolyte *put with* GREEN WEED.	6 with RED-BROWN WEED.
Record on July 19	1 pale green. 2 green liners. 1 reddish. 1 transparent. [1 eaten.]	1 pale brown. 1 brownish liner. [4 dead.]
„ July 21	1 pale green. 1 sage green. 1 red liner. [Rest eaten.]	1 pale reddish-brown, 1 centim. long (*i.e.*, grown ÷ 4 millims.). [Rest dead.]
	Microscopical examination :— Pale green specimen—♂. Chromatophores—evenly distributed over body. Pigments— Yellow—well developed, +. Red—small amount. Blue—fair amount.	Microscopical examination :— Pale reddish-brown specimen—♀. Chromatophores fairly evenly distributed, but specially developed in mid-dorsal and ventral lines. Pigments— Red and yellow—well developed, +. Blue—small in amount, +.

TABLE IV.—*Sympathetic Colour-change—Green to Brown. Hippolyte varians.* TRÉGASTEL, 1904. Animals used in the experiment: *young*, 6–8 millims., *and had become green on green weed.* July 29, eight *Hippolyte* transferred from green to brown weed.

July 30.	August 1.	Colour of animals.
—	3 (matching weed)	Greenish-brown.
1	—	Yellow-brown.
1	—	Yellow.
—	1	Grey.
[1]	[2]	[Eaten.]
3 brown about gills	2	Transparent.
2	—	Green.

The table shows that of 8 young *Hippolyte* which had become green on green weed, 3 out of 6 survivors became greenish-brown on brown weed in three days.

TABLE V.—*Sympathetic Colour-change* in *Mid-sized Hippolyte varians*. TRÉGASTEL, 1904. July 29, put *mid-sized red-lined forms on green weed* (*Zostera*).

July 30.	July 31.	August 1.	August 2.	August 3.	August 6.	August 9.	Colour of animals.
—	—	1	1	—	4	4	Green (liner).
—	—	The rest all recognisable as red-liners of dull tint.		3	—	—	Dull green (liner).
—	—			1	—	—	Yellow-green (liner).
—	—			2	2	2	Yellow speckled ,,
—	—			—	—	—	Red ,, ,,
12	12			6	6	6	Red (liner).

The table shows that of 12 red-striped half-grown *Hippolyte*, 4 became green on green weed in a week.

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EXPLANATION OF PLATES.

Plate 1.

Figs. 1–6 illustrate the development of the chromatophores of the common shrimp. The pigments are omitted. For an account of their distribution and development, see p. 5. The preparations were fixed in corrosive-acetic sublimate and stained with Heidenhain's hæmatoxylin.

Figs. 7–12 illustrate the development of the chromatophores of *Hippolyte varians*. Of these, figs. 7, 8, and 10 are taken from immature specimens, fixed with formalin-acetic, and stained with Heidenhain's hæmatoxylin. Fig. 9 is from the carapace of an adult prawn, and shows the central branched cell that contains the diurnal blue and the fat. Fig. 11 is another chromatophore from an adult specimen, fixed with osmic acid. Fig. 12 is an intermuscular chromatophore showing its connection with a neighbouring centre.

Plate 2.

Figs. 13–17 illustrate the development of the chromatophores of *Hippolyte gaimardii*, and are all taken from adult specimens. The two pigments, red and yellow, are omitted.

Figs. 18–20 show the movements of the colourless fat in the chromatophores of *Hippolyte varians*. Yellow pigment is indicated by short strokes, red by hatching. The fat appears to be in the cell that also contains the diurnal blue pigment.

Fig. 21 shows the depletion of fat and the destruction of pigment in a chromatophore of *Hippolyte varians* kept in the dark without food for eight days: x spaces left by destruction of pigment.

All the figures are magnified 550 times, and are taken from camera drawings.

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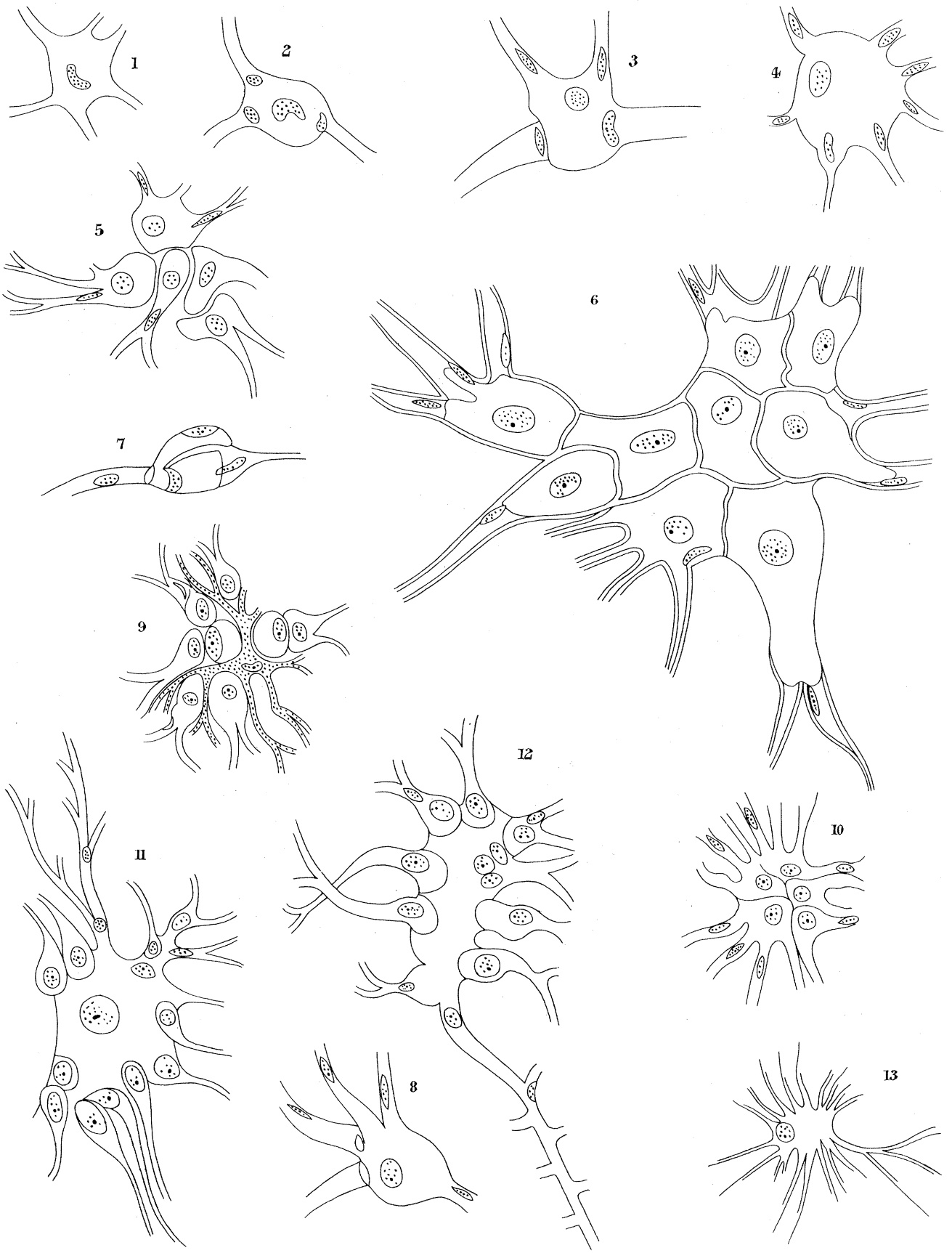

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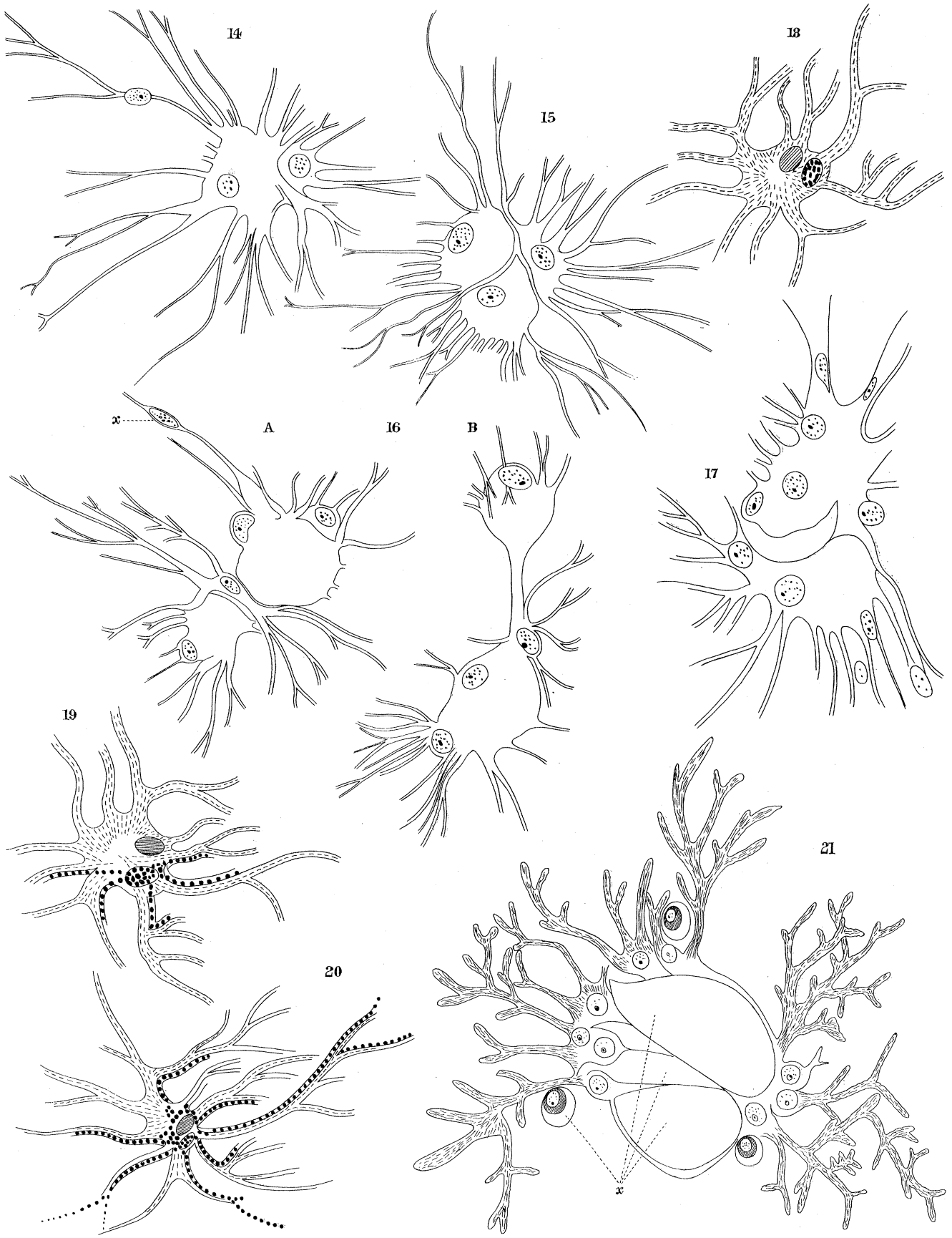

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